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DESCRIPTION

PLANT TISSUE TREATMENT COMPOSITION

Technical Field

The present invention relates to a composition for use in treating fibers.

Background Art

Cellulose fibers typically represented by cotton fiber are composed of a secondary film comprising cellulose and a primary film covering the secondary film through a winding layer and comprising wax, pectin and protein. The procedure for removing the primary film for the purpose of improving the absorptivity, decolorability and dyeability of fibers is generally referred to as scouring.

For scouring, conventionally, a method has been used, comprising immersing fiber in a mixture solution of sodium hydroxide and a scouring auxiliary agent containing a surfactant and treating the fiber at a high temperature However, the method is disadvantageous in that the method requires a vast amount of energy and is

costly because liquid waste of strong alkalinity should be treated.

So as to overcome the problems, a new scouring method by means of enzymes (Japanese Patent Laid-open No.220772/1994) has been proposed, and protopectinase is used according to the method.

Protopectinase is the generic name of enzymes releasing pectin from plant fibers. It is described that protopectinase has a wide variety of usefulness in the treatment of plant tissues, including for example the production of pectin useful as a raw material of food, drugs and cosmetics (Japanese Patent Publication No. 8322/1994) and the pulping of non-wood fiber (Japanese Patent Publication No 39636/1983). Protopectinase is broadly classified as the following two types. One is type A, which is generated by enzymes to release pectin from plant fibers and which has also an activity to decompose free pectin in a limiting manner (Japanese Journal of Fermentation and Industry, 37, 928 - 938, 1978). The other is type B, which is generated by bacteria of genus Bacillus to release pectin from plant fibers but which never has any activity to decompose free pectin (Agri. Biol. Chem., 52, 1091 - 1093, 1988; Agric. Biol. Chem., 53, 1213 - 1223, 1989; Agric. Biol. Chem., 54, 879 - 889, 1990;

Eur. J. Biochem., 226, 285 - 291, 1994). It is the latter type B that has particularly great usefulness in scouring fibers.

As supply sources of protopectinase, conventionally, use has been made of culture broths of a microorganism generating protopectinase, of themselves. From the standpoint of procedure, however, the use of the culture broths for scouring fibers is disadvantageous. In terms of the production cost of enzymes, a method capable of scouring fibers at lower cost has been desired for industrial application. If the problem can be overcome, furthermore, the industrial application of such enzymes to other utilities such as pulp production and pectin production will also be expected.

Therefore, the present invention described in this specification realizes the application of a composition enhancing the potency of an enzyme to treat plant tissues.

Disclosure of the Invention

In accordance with the present invention, an arabinase with the following properties is provided;

(1) having an arabinan decomposition activity;

(2) having a molecular weight of about 65,000 on gels

by SDS - polyacrylamide electrophoresis;

(3) having an optimum pH of 7.0;

(4) having an optimum temperature of 50 degrees; and

(5) being inhibited with Ag, Cd, Cu, Hg, Mn and Zn.

In one embodiment, the arabinase is generated by Bacillus subtilis strain BS.

In one embodiment, the arabinase has an amino acid sequence of SEQ ID NO.1 or the arabinase has an amino acid sequence through deletion, substitution or addition of one or several amino acids from the amino acid sequence and has an activity at the same level as or a higher level than the level of the arabinase having the amino acid sequence of SEQ ID NO.1.

In accordance with the present invention, a composition containing the arabinase and protopectinase is provided.

In one embodiment, the composition additionally contains a soy bean powder extract solution.

In one embodiment, the protopectinase in the composition is a protopectinase having the following properties;

(1) having a protopectin decomposition activity;

(2) having a molecular weight of about 30,000 on gels by SDS - polyacrylamide electrophoresis;

(3) having an optimum pH of 6.0;

(4) having an optimum temperature of 60 degrees; and

(5) being inhibited with Hg and Mn.

In one embodiment, the protopectinase in the composition is generated by Bacillus subtilis strain BS.

In accordance with the present invention, a method for preparing a composition containing the arabinase and protopectinase is provided, comprising a process of culturing a microorganism of genus Bacillus, the microorganism generating the arabinase and protopectinase, and a process of recovering a fraction containing the arabinase and protopectinase from the culture broth.

In accordance with the present invention, a method for scouring fibers comprising reacting the composition with fibers is provided.

In accordance with the present invention, a method for producing pectin comprising reacting the composition with fibers is provided.

In accordance with the present invention, a method for producing pulp comprising reacting the composition with fibers is provided.

In accordance with the present invention, a DNA coding for the arabinase is provided.

In one embodiment, the DNA has the nucleotide sequence of SEQ ID NO.2 or the DNA has a nucleotide

sequence generated through deletion, substitution or addition of one or several nucleotides from the nucleotide sequence and the DNA codes for an arabinase with an activity at the same level as or at a higher level than the level of the activity of the arabinase encoded by the nucleotide sequence of SEQ ID NO.2.

In accordance with the present invention, an expression vector carrying the DNA is provided.

In accordance with the present invention, a host cell transformed with the expression vector is provided.

In accordance with the present invention, it is provided a method for producing an arabinase, comprising a process of culturing a microorganism with the potency of generating the arabinase with the following properties;

(1) having an arabinan decomposition activity;

(2) having a molecular weight of about 65,000 on gels by SDS - polyacrylamide electrophoresis;

(3) having an optimum pH of 7.0;

(4) having an optimum temperature of 50 degrees; and

(5) being inhibited with Ag, Cd, Cu, Hg, Mn and Zn.

In one embodiment, the microorganism according to the production method is Bacillus subtilis strain BS.

In one embodiment, the production method is a method for producing an arabinase, comprising a process

of culturing a host cell transformed with the expression vector, wherein the arabinase has the following properties;

(1) having an arabinan decomposition activity;

(2) having a molecular weight of about 65,000 on gels by SDS - polyacrylamide electrophoresis;

(3) having an optimum pH of 7.0;

(4) having an optimum temperature of 50 degrees; and

(5) being inhibited with Ag, Cd, Cu, Hg, Mn and Zn.

In accordance with the present invention, it is provided a scouring composition containing the arabinase, the protopectinase and a soy bean powder extract solution.

In accordance with the present invention, it is provided a method for scouring fibers is provided, comprising a process of reacting an enzyme solution containing the arabinase and the protopectinase with fibers.

In one embodiment, a soy bean powder extract solution is further contained according to the method for scouring fibers.

Brief Description of Drawings

Fig.1 shows graphs depicting the effect of protopectinase C on the activity of arabinase when

arabinase BS is added;

Fig.2 shows graphs depicting the effect of protopectinase C on the activity of protopectinase when arabinase BS is added;

Fig.3 shows graphs depicting the effects of arabinase BS and protopectinase C on the activity of arabinase when a soy bean powder extract solution is added;

Fig.4 shows photographs of cotton yarn derived from non-treated cotton cloth by field emission (FE) - type scanning electron microscope;

Fig.5 shows photographs of cotton yarn derived from cotton cloth after pectin removal treatment by field emission (FE) - type scanning electron microscope;

Fig.6 shows photographs of cotton yarn derived from cotton cloth after pectin removal treatment by field emission (FE) - type scanning electron microscope;

Fig.7 shows photographs of linen yarn derived from non-treated linen cloth by field emission (FE) - type scanning electron microscope;

Fig.8 shows photographs of linen yarn derived from linen cloth after pectin removal treatment by field emission (FE) - type scanning electron microscope;

Fig.9 shows photographs of linen yarn derived from non-treated linen cloth by field emission (FE) - type

scanning electron microscope; and

Fig.10 shows photographs of linen yarn derived from linen cloth after pectin removal treatment by field emission (FE) - type scanning electron microscope.

Best Mode for Carrying out the Invention

Cellulose fibers typically represented by cotton fiber are composed of a secondary film comprising cellulose and a primary film covering the secondary film through a winding layer and comprising wax, pectin and protein. The procedure for removing the primary film for the purpose of improving the absorptivity, decolorability and dyeability of fibers is generally referred to as scouring. The scouring of the present invention includes the treatment of non-lignocellulose fibers such as linen for the same purpose. Preferably, scouring is carried out by a method using enzymes such as protopectinase. The assessment of scouring process may be practiced by the measurement of free pectin, the measurement of the absorptivity of fiber or the measurement of the strength thereof such as elongation and compressibility, or the observation of the surface under an electron microscope or at an organoleptic test.

Pectin is the generic name of acid polysaccharides contained the cell wall of plants, and is utilized as a

raw material of food, drugs and cosmetics. Pectin may be extracted in the presence of chelating agents or strong acids or strong alkalis by thermal extraction from plant tissues. Preferably, pectin is released through the action of enzymes such as protopectinase.

Pulp is the suspension of plant cellulose fibers, and is utilized as a raw material of paper and artificial fibers. Pulp may be produced by treating plants such as wood, mechanically or chemically. Preferably, pulp is produced by treating non-wood fibers with enzymes such as protopectinase.

Protopectinase means an enzyme with an activity to release water-soluble pectin from insoluble protopectin in plant tissues. Protopectinase is classified as type A with an activity to decompose free pectin in a limiting manner and type B with no activity to decompose free pectin. Protopectinase activity may be determined by reacting an enzyme solution with protopectin as the substrate and assaying released pectin in a colorimetric manner.

Arabinase means an enzyme with an activity to react with arabinan to release arabinose. Arabinase activity may be determined by reacting an enzyme solution with arabinan as the substrate and assaying released arabinose with the Nelson - Somogyi method. The

molecular weight of an enzyme may be determined on the basis of the mobility on a gel for SDS polyacrylamide electrophoresis or the performance on a gel filtration column. The optimum pH or optimum temperature of an enzyme may be determined by assaying the enzyme activity at a variety of pHs or temperatures. A substance with some influence on the enzyme action may be identified by adding a variety of substances into a reaction solution during enzyme reaction.

Protopectinase or arabinase may be produced by culturing a microorganism producing these enzymes or by culturing a host cell with an expression vector incorporated therein, the vector carrying a DNA coding for these enzymes. As a microorganism to produce these enzymes, for example, use is made of bacteria of genus Bacillus. More preferably, use is made of Bacillus subtilis strain BS. For producing these enzymes by genetic recombination technology, an optional host cell for general use may be used, but preferably, use is made of microorganisms such as Escherichia coli or Bacillus subtilis.

The Bacillus subtilis strain BS has been referred to as Bacillus subtilis strain IFO3134 previously. The strain is characteristic in that the strain is Gram positive, having spore formation potency, being a

bacillus bacterium, having low protease production potency, and requiring phosphate ion for producing an active substance enzyme (namely, arabinase and/or protopectinase), wherein the production of the enzyme is inhibited by glucose. The microorganism is deposited as Accession No. FERM BP-6031 on date July 30, 1997 at the Life Engineering and Industrial Technology Research Institute, the Agency of Industrial Science and Technology, the Ministry of Trade and Commerce, at 1 - 3, Higashi 1-chome, Tsukuba-shi, Ibaraki-ken, Japan (Zip code 305).

The cloning of a gene coding for arabinase may be practiced by preparing a library containing the chromosomal DNA extracted from a microorganism producing the enzyme, and screening the library. The screening may be conducted by expressing an encoded protein and using the activity of the objective protein or the reactivity thereof with a specific antibody against the protein as a marker. Otherwise, the screening may be conducted by using a labeled probe designed on the basis of the amino acid sequence of a purified protein. Preferably, the labeled probe is prepared by using a fragment generated through polymerase chain reaction (PCR) wherein use is made of a primer designed on the basis of the amino acid sequence of the purified

protein. The cloning of the arabinase gene may also be achieved by combining together fragments produced by PCR.

As the expression vector, those with compatibility with a host cell to be used are selected. Generally, a multicopy vector with a strong promoter is used. When the transcription efficiency of a promoter which the objective gene essentially has is high, the promoter may be utilized. When the expression of the objective gene affects the proliferation of the host cell, an inductive promoter is preferably used.

Microorganisms are cultured under appropriate conditions for the microorganisms to be used. Any of solid culture media and liquid culture media for routine use may be used. Culturing is carried out under aerobic conditions or anaerobic conditions, depending on a microorganism to be used. When a microorganism is cultured under aerobic conditions, aeration-agitation- or shaking culture may be conducted. When a bacterium of genus Bacillus is used, the culturing is preferably performed by using a liquid culture medium under aerobic conditions. The ingredients of the culture medium are not limited, but use may be made of a culture medium containing an appropriate combination of carbon sources, nitrogen sources, phosphate and the like, bacterially

assimilable, at appropriate concentrations, for the proliferation of the microorganism and the production of substances. Preferably, a culture medium containing a soy bean powder with an effect to induce the production of protopectinase and arabinase is used. The culture duration is determined, depending on the timing of the production of a substance to be produced and the properties of a microorganism to be used, but generally, the duration is preferably about 16 to 48 hours.

The purification of enzymes is conducted as follows. When the objective enzyme is secreted out of bacteria, the bacteria are removed through centrifugation or filtration from the culture broth, to recover the supernatant. When the objective enzyme is accumulated inside bacteria, the bacteria are recovered from the culture broth, which are treated with lyases or ultrasonication, followed by conducting centrifugation and the like, to recover a cell-free extract solution. By a combination of routine protein purification means, for example concentration by drying or ultra-filtration, precipitation by salting out or solvent precipitation, dialysis, gel filtration chromatography or ion exchange chromatography, a purified enzyme can be recovered from such solution.

When both arabinase and protopectinase are

contained in a single culture broth, the two types of enzymes can be separated from each other by chromatography procedures on Butyl-Toyopearl 550M and the like.

The soy bean powder extract solution means a liquid extracted from the powder of soy bean. As soy bean, preferably, use is made of defatted soy bean. Extraction may be done by subjecting a solution containing soy bean powder to thermal treatment in the presence of caustic sodium by means of autoclave.

The composition of the present invention contains protopectinase and arabinase. The ratio of protopectinase and arabinase depends on the utility thereof, with no specific limitation. Generally, these enzymes may be used at a ratio of 1:9 to 9:1, preferably 3:7 to 7:3, and more preferably 4:6 to 6:4, on an arabinase activity basis. These enzymes may also be used at a ratio of 1:1. Due to the presence of both the enzymes, a synergistic effect develops, whereby the activity can be enhanced. When the composition is used for scouring, therefore, a higher activity can be yielded at a lesser enzyme amount than conventionally.

Furthermore, the composition of the present invention may contain a soy bean powder extract solution. The soy bean powder extract solution may be

added at 0.01 % to 0.3 %, desirably 0.02 % to 0.1 %, and more desirably 0.02 % to 0.05 % into the composition. The activity of protopectinase or arabinase may further be enhanced due to the soy bean extract solution contained therein. Hence, a higher activity can be recovered at a lesser enzyme amount than conventionally, if the composition is used for scouring and the like.

Then, scouring is practiced by using the composition of the present invention. Scouring is practiced by adding Warming TE (manufactured by Tokai Chemical) into a buffer and heating then the buffer, immersing fiber or cloth in the buffer and keeping the resulting buffer warm, and thereafter adding the composition containing the enzymes into the buffer followed by shaking and agitation.

The scouring may be assessed by assaying the remaining pectin and/or wax or assaying the whiting degree.

The present invention will now be described in detail in examples hereinbelow. However, the present invention is not limited to these examples.

(Example 1: Purification of protopectinase and arabinase)

1.1 Purification of protopectinase

The activity of protopectinase was assayed by using protopectin of sugar beet pulp as the substrate, according to the method by Sakai et. al. (Methods in Enzymology, 161, 335 - 350, 1988, edited by W. A. Wood and T. Kellogg, Academic Press) as described below; 10 microliter of an enzyme solution was added into an acetate buffer (100 mM, pH 6.0) of 990 microliter, containing 10 mg of the substrate, and the resulting mixture was incubated at 37 degrees for one hour, to filtrate the reaction solution; 125 microliter of the filtrate, 125 microliter of water, 250 microliter of ethanol containing 0.2 % carbazol and 3 ml of 31.5 N sulfuric acid were mixed together and incubated at 75 degrees for 20 minutes. After ice cooling the reaction solution, the absorbance was measured at 525 nm. Protopectinase activity was calculated on the basis of the relation formula between the amount of galacturonic acid and the color development degree, provided that one unit of protopectinase was defined as the activity to produce pectin corresponding to 1 micromole galacturonic acid per one minute.

Bacillus subtilis strain BS was inoculated on an SP culture medium (1.5 % soy bean powder (Fuji Oil Industry), 1.2 % KH_2PO_4, 2.8 % K_2HPO_4, pH 7.0), prior to agitation culture at 37 degrees for 22 hours. The

culture broth was centrifuged to recover the supernatant. The supernatant was concentrated with a rotary evaporator, and subsequently, the concentrate was dialyzed at 4 degrees overnight against 20 mM acetate buffer (pH 6.0). From the dialyzed enzyme solution was recovered a fraction saturated with 0 to 60 % ammonium sulfate, and the precipitate was dissolved in 20 mM Tris-HCl (pH 7.5), and the resulting solution was dialyzed at 4 degrees overnight against the same buffer.

Ammonium sulfate was added into the dialyzed enzyme solution to a final concentration of 30 %, and the resulting mixture was applied to a Butyl-Toyopearl 650 M column (manufactured by Toso) equilibrated with 20 mM Tris-HCl (pH 7.5) containing 30 % ammonium sulfate. After sufficient rinsing of the column with the same buffer and elution on a continuously linear concentration gradient of 30 to 10 % saturated ammonium sulfate, fractions with protopectinase activity were separated and collected. Then, the fractions were applied to a Superdex 75 (manufactured by Pharmacia Biotech) column equilibrated with 20 mM acetate buffer (pH 6.0) containing 100 mM NaCl. Through elution with 20 mM acetate buffer (pH 6.0) containing 100 mM NaCl, a fraction with protopectinase activity was fractionated and collected. On a gel for polyacrylamide

electrophoresis (abbreviated as SDS - PAGE hereinafter), the resulting enzyme solution was observed to be present on a single band, which was used as a purified enzyme sample at the following procedures. The purification (process) was collectively shown in Table 1.

Table 1 Purification of protopectinase

Procedures	Total protein (mg)	Total activity (Unit)	Specific activity (units/mg)	Purification degree (fold)	Yield (%)
Culture broth	47,880	277,200	5.8	-	100
Ammonium sulfate precipitation	1,900	151,900	79.9	13.8	54.8
Butyl-Toyopearl 650M	40.0	2,620	65.5	11.3	0.95
Superdex 75	15.3	1,950	127	21.9	0.70

1.2 Purification of arabinase

L-Arabinan to be used as a substrate for the assay of arabinase activity was prepared as follows; sugar beet pulp was treated in 0.1N NaOH at 100 degrees for one hour; after the treatment, the resulting matter was filtered and centrifuged, and the resulting solution was mixed with ethanol of a 3-fold volume, and the resulting

precipitate was collected through centrifugation; the precipitate was dissolved in water, followed by centrifugation to remove the insoluble matter, and the resulting supernatant was dialyzed against 50 mM acetate buffer (pH 5.0) under the action of galactanase (100 units/ml), at 37 degrees for 24 hours; the dialyzed solution was applied to a DEAE-Cellulofine AH column (manufactured by Toso) equilibrated with 50 mM acetate buffer (pH 6.0), to isolate and collect the non-adsorbed fraction, which was then dialyzed against water and mixed with ethanol of a 3-fold volume, and the resulting precipitate was rinsed twice in ethanol, followed by freeze- drying.

The arabinase activity was assayed as follows; an enzyme solution of 10 microliter was added into 190 microliter of 100 mM acetate buffer (pH 7.0) containing L-arabinan prepared as described above at 0.5 %, for incubation at 50 degrees for 10 minutes; 200 microliter of Somogyi reagent was added into the incubation mixture, followed by boiling for 10 minutes, and the resulting mixture was cooled in ice for 10 minutes; 200 microliter of Nelson reagent was added into the mixture, which was then left to stand at room temperature for 20 minutes, to measure the absorbance at 660 nm. Arabinase activity was calculated and expressed on the basis of a

formula about the relation between the amount of arabinose and the color development degree, provided that one unit of arabinase activity was defined as an activity releasing reduced sugar at the amount corresponding to 1 micromole arabinose per one minute.

As to the purification of arabinase, the process from the culturing of microorganisms to the procedure on a column Butyl-Toyopearl 650M was conducted in the same manner as for protopectinase. Fractions with arabinase activity as eluted from the Butyl-Toyopearl 650 M column were dialyzed against 20 mM Tris-HCl (pH 7.5), and to the resulting dialyzed solution was added ammonium sulfate to a final concentration of 30 %, and the resulting mixture was again applied to a column Butyl-Toyopearl 650 M under the same conditions as in Example 1, to isolate and collect fractions with arabinase activity. Then, the fractions were applied to a Superdex 75 column equilibrated with 20 mM acetate buffer (pH 6.0) containing 100 mM NaCl. Through elution with 20 mM acetate buffer (pH 6.0) containing 100 mM NaCl, fractions with arabinase activity were isolated and collected. Then, the fractions were applied to a column Superdex 200 (manufactured by Pharmacia Biotech) equilibrated with 20 mM acetate buffer (pH 6.0). Through elution with 20 mM acetate buffer (pH 6.0), a

fraction with arabinase activity was isolated and collected. The resulting enzyme solution was observed to have a single band on a gel by polyacrylamide electrophoresis (abbreviated as SDS-PAGE hereinbelow), which was then used as a purified enzyme sample at the following procedures. The purification was collectively shown in Table 2

Table 2 Purification of arabinase

Procedures	Total protein (mg)	Total activity (unit)	Specific activity (units/mg)	Purification degree (fold)	Yield (%)
Culture broth	47,880	182,000	3.8	-	100
Ammonium sulfate precipitation	1,900	125,000	65.8	17.3	68.7
Butyl-Toyopearl 650M	58.8	5,510	93.7	24.7	3.0
Butyl-Toyopearl 550M	16.5	4,570	277	72.9	2.5
Superdex 75	7.5	2,630	350	92.1	1.4
Superdex 75	1.2	590	492	129	0.3

(Example 2: Characterization of protopectinase)

2.1 Molecular weight

The molecular weight of protopectinase was estimated on the basis of the mobility on 10 % SDS-PAGE gel, which was about 30,000. When the enzyme was applied to a Superdex 75 gel filtration column equilibrated with 20 mM acetate buffer (pH 6.0) containing 100 mM NaCl, the molecular weight was estimated as about 27,000.

2.2 pH and temperature stability

Protopectinase was incubated at a concentration of 5 units/ml in 100 mM acetate buffer (pH 7.0) containing 50 microgram/ml bovine serum albumin, at a variety of pHs and at 37 degrees for one hour, and then, the enzyme activity was assayed by the method described in Example 1. 80 % or more of the activity remained at pH 6 to 9. Protopectinase was incubated at a concentration of 5 units/ml in 100 mM acetate buffer (pH 7.0) containing 50 microgram/ml bovine serum albumin, at a variety of temperatures, for 30 minutes, and then, the enzyme activity was assayed. 80 % or more of the activity remained at a temperature below 50 degrees.

2.3 Optimum pH and optimum temperature

The protopectinase activity was assayed by the same method as in Example 1, except for the modification of the reaction pH and the reaction temperature. The maximum activity was observed at pH 6.0 and 60 degrees.

2.4 Effects of a variety of ions

At pH 6.0 and 50 degrees was assayed protopectinase activity in reaction solutions containing a variety of substances described in Table 3 at a concentration of 1 mM, and the activity was completely inhibited in the

presence of $HgCl_2$, while 75 % of the activity was inhibited in the presence of $MnCl_2$.

Table 3 Effects of ions on protopectinase activity

Name of substance	Relative activity (%)
None	100
$AgNO_2$	60
$BaCl_2$	100
$CaCl_2$	95
$CdCl_2$	85
$CoCl_2$	105
$CuSO_4$	90
$FeCl_2$	105
$HgCl_2$	0
KCl	100
$MgCl_2$	100
$MnCl_2$	25
NaCl	100
$NiCl_2$	95
$ZnCl_2$	90
NaF	95
NaN_2	105
$NaNO_2$	80
p-Chlorobenzoic acid mercuric	110
EDTA	90

2.5 Substrate specificity

Protopectinase reacted with a variety of

substrates, which exerted protopectinase activity on protopectin (derived from sugar beet) and L-arabin(an?) (derived from sugar beet) and also exerted arabinase activity on arabinogalactane (derived from soy bean). The arabinase activity was assayed as follows; an enzyme solution of 10 microliter was added into 190 microliter of 100 mM acetate buffer (pH 7.0) containing L-arabinan at 0.5 %, for incubation at 50 degrees for 10 minutes; 200 microliter of Somogyi reagent was added into the incubation mixture, followed by boiling for 10 minutes, and the resulting mixture was cooled in ice for 10 minutes; 200 microliter of Nelson reagent was added into the mixture, which was then left to stand at room temperature for 20 minutes, to measure the absorbance at 660 nm. Arabinase activity was calculated and expressed on the basis of a formula about the relation between the amount of arabinose and the color development degree, provided that one unit of arabinase activity was defined as an activity releasing reduced sugar at the amount corresponding to 1 micromol arabinose per one minute. L-arabinan to be used as the substrate was prepared as follows; sugar beet pulp was treated in 0.1N NaOH at 100degrees for one hour; and after the treatment, filtration and centrifugation were effected, the resulting solution was mixed with ethanol of a 3-fold

volume, and then, the resulting precipitate was collected through centrifugation; the precipitate was dissolved in water, followed by centrifugation to remove the insoluble matter, and the resulting supernatant was dialyzed against 50 mM acetate buffer (pH 5.0) under the action of galactanase (100 units/ml) at 37 degrees for 24 hours; the dialyzed solution was applied to a DEAE-Cellulofine AH column (manufactured by Toso) equilibrated with 50 mM acetate buffer (pH 6.0), to pool non-bound fractions, which were then dialyzed against water and then mixed with ethanol of a 3-fold volume; and the resulting precipitate was rinsed twice in ethanol, followed by freeze- drying.

The enzyme never reacted with the remaining substrates. The assay of other activities was done at pH 7.0, according to the method by F. M. Rombouts et. al. [Carbohydrate Polymers, Vol.9, page 25 - 47 (1988)].

Based on the above results, it was verified that the protopectinase of the present invention is a novel protopectinase different from conventionally known protopectinase. Thus, the protopectinase was designated as protopectinase C.

Table 4 Protopectinase activity on a variety of substrates

Activity	Substrate (origin)	Presence or absence of activity
Protopectinase	protopectin (sugar beet)	presence
	L-arabinan (sugar beet)	presence
Arabinase	arabinogalactane (soy bean)	presence
	arabinogalactane ()	absence
Galactanase	beta-1,4-galactane (soy bean)	absence
Xylanase	beta-1,4-xylane (subert wheat)	absence
Cellulase	CM-cellulose	absence
Pectin lyase	highly methoxylated polygalacturonic acid (orange)	absence
Pectate lyase	polygalacturonic acid (orange)	absence
Endo-polygalacturonase	polygalacturonic acid (orange)	absence
Exo-polygalacturonase	polygalacturonic acid (orange)	absence
beta-D-Galactosidase	D-nitrophenyl-beta-D-galatoside	absence
alpha-D-Arabinofuranosidase	p-nitrophenyl-alpha-L-arabinofuranoside	absence

alpha-L-Arabinopyranosidase

p-nitrophenyl-alpha-L-arabinopyranoside

absence

2.6 Amino acid sequence of protopectinase

The amino acid sequence of purified protopectinase at the N-terminus of the mature protein was analyzed, to recover the amino acid sequence of SEQ ID NO.6. By using GenBank, EMBL and SWISS-PROT data bases to screen a protein with homology, no known protein with the same sequence was found, whereby it was confirmed from the amino acid sequence that the protopectinase thus recovered was novel.

(Example 3: Characterization of arabinase)

2.1 Molecular weight

The molecular weight of arabinase was estimated on the basis of the mobility on 10 % SDS-PAGE gel, which was about 65,000. When the enzyme was applied to a Superdex 200 gel filtration column equilibrated with 20 mM acetate buffer (pH 6.0) containing 100 mM NaCl, the molecular weight was estimated as about 70,000.

2.2 pH and temperature stability

Arabinase was incubated at a concentration of 5

units/ml in 100 mM acetate buffer (pH 7.0) at a variety of pHs and at 37 degrees for one hour, and then, the enzyme activity was assayed by the method described in Example 2. 80 % or more of the activity remained at pH 7 to 10. Arabinase was incubated at a concentration of 5 units/ml in 20 mM acetate buffer (pH 6.0) at a variety of temperatures for 30 minutes, and then, the enzyme activity was assayed. 80 % or more of the activity remained at a temperature below 60 degrees.

2.3 Optimum pH and optimum temperature

The arabinase activity was assayed by the same method as in Example 2, except for the modification of the reaction pH and the reaction temperature. The maximum activity was observed at pH 7.0 and 50 degrees.

2.4 Effects of a variety of ions

At pH 6.0 and 50 degrees was assayed arabinase activity in reaction solutions containing a variety of substances described in Table 5 at a concentration of 1 mM, and the activity was completely inhibited in the presence of $AgNO_3$, $CuSO_4$, $HgCl_2$, and $ZnCl_2$, while 95 % of the activity was inhibited in the presence of $CdCl_2$.

Table 5 Effects of ions on arabinase activity

Name of substance	Relative activity (%)
None	100
$AgNO_3$	0
$BaCl_2$	100
$CaCl_2$	95
$CdCl_2$	5
$CoCl_2$	106
$CuSO_4$	0
$FeCl_2$	75
$HgCl_2$	0
KCl	85
$MgCl_2$	110
$MnCl_2$	15
NaCl	90
$NiCl_2$	70
$ZnCl_2$	0
NaF	95
NaN_2	85
$NaNO_2$	98
Mercuric p-chlorobenzoate	65
EDTA	85

2.5 Substrate specificity

Arabinase reacted with a variety of substrates,

which exerted protopectinase activity on protopectin (derived from sugar beet) and L-arabin (derived from sugar beet) and exerted arabinase activity on arabinogalactane (derived from soy bean) and alpha-L-arabinofuranosidase activity on p-nitrophenyl-alpha-L-arabinofuranoside, but arabinase did not react with other substrates. The alpha-L-arabinofuranosidase activity and other activities were assayed at pH 7.0, according to the method described by F. M. Rombouts et al., Carbohydrate Polymers, Vol.9, page 25-47 (1998)

Table 6 Arabinase activity on a variety of substrates

Activity	Substrate (origin)	Presence or absence of activity
Protopectinase	protopectin (sugar beet)	absence
	L-arabinan (sugar beet)	presence
Arabinase	arabinogalactane (soy bean)	presence
	arabinogalactane (Japanese larch)	absence
Galactanase	beta-1,4-galactane (soy bean)	absence
Xylanase	beta-1,4-xylane (subert wheat)	absence
Cellulase	CM-cellulose	absence
Pectin lyase	highly methoxylated polygalacturonic acid (orange)	absence
Pectate lyase	polygalacturonic acid (orange)	absence
Endo-polygalacturonase	polygalacturonic acid (orange)	absence

Exo-polygalacturonase

polygalacturonic acid (orange)

absence

beta-D-Galactosidase

D-nitrophenyl-beta-D-galatoside

absence

alpha-D-Arabinofuranosidase

p-nitrophenyl-alpha-L-arabinofuranoside

presence

alpha-L-Arabinofuranosidase

p-nitrophenyl-alpha-L-arabinopyranoside

absence

Based on the aforementioned results, it was verified that the arabinase of the present invention was a novel arabinase, different from conventionally known arabinase. The arabinase was designated as arabinase BS.

(Example 4: Determination of partial amino acid sequence of arabinase BS)

4.1 Determination of N-terminal amino acid sequence

13 microgram of the purified arabinase BS was reduced with 5 % mercaptoethanol, followed by separation

by 10 % polyacrylamide electrophoresis. The separated protein in a blotting solution (10 % methanol/10 mM CAPS/NaOH (pH 10.5)) was blotted electrically (100 V, 1.5 hr) on a PVDF membrane (Immobilon PSEQ, manufactured by Millipore, Co.). After rising the PVDF membrane in distilled water, the membrane was stained with 0.1 % Coomassie Blue R-250/50 % methanol. By decolorability the membrane by using 50 % methanol, a band corresponding to the arabinase BS stained was excised, which was then rinsed in distilled water, to identify the N-terminal amino acid sequence by using Applied Biosystems model 492. The identified N-terminal amino acid sequence of arabinase BS was TDIVKGTINKNIYGHFAEHL.

4.2 Identification of partial amino acid sequence

32.5 microgram of the purified arabinase BS was reduced in 8 M urea/0.5 M Tris-HCl (pH 8.0) solution with 50 mM dithiothreitol at 37 degrees for 6 hours, continuously followed by incubation in 62.5 mM ICH_2COONa at ambient temperature for 5 minutes, to reduce and carboxymethylate the arabinase BS. The resulting carboxymethylated sample was dialyzed against 50 mM Tris-HCl (pH 8.0), and was then digested with 400 ng of lysyl endopeptidase (manufactured Wako Pure Chemical Industry, Co.) at 37 degrees for 16 hours. The

resulting peptide fragments were fractionated by reverse-phase HPLC by using Chemcosorb 3 ODS-H (manufactured by Chemco Co.). By using Applied Biosystems Model 492, the amino acid sequence was determined. Among the fractionated peptide fragments, six fragments were identified of their N-terminal amino acid sequences, and the results are shown in the following Table 7.

Table 7 N-terminal amino acid sequences of lysyl endopeptidase-digested fragments of arabinase BS

Peptide No.	Amino acid sequence
No.1	NIYGHFAEHL
No.2	LTYFGVGNENWGCGGNMHPEYYADLYR
No.3	YIDELIQK
No.4	AEAEIELRGLHK
No.5	AADHSGIILTADK
No.6	MNAHNTFDDPHHVKPESFSQ

(Example 5: amplification of arabinase BS gene fragments by PCR)

5.1 Preparation of genome DNA

The genome DNA of Bacillus subtilis strain BS was prepared by using CTAB, according to Molecular Cloning, 3-rd edition (1993).

5.2 Preparation of primers

So as to amplify partial fragments of arabinase BS gene using the genome DNA as the template by PCR, the following two types of degenerated oligonucleotide primers (BS-1 and BS-2) were synthesized on the basis of the partial amino acid sequences identified in Example 4, by using an automatic DNA synthesizer. The primer BS-1 was designed as a nucleotide sequence corresponding to the partial sequence (Asn-Ileu-Tyr-Gly-His-Phe-Ala) of the N-terminal amino acid sequence of the arabinase BS determined in Example 4. The primer BS-2 was designed as the complementary chain to a nucleotide sequence corresponding to the partial amino acid sequence (Ala-His-Asn-Thr-Phe-Asp-Asp) of the lysyl endopeptidase fragment No.6 of the arabinase BS as determined in Example 4.

Primer BS-1 (SEQ ID No.4)

Primer BS-2 (SEQ ID No.5)

Herein, R represents G or A; Y represents T or C; H represents A, C or T; and N represents A, C, G or T.

5.3 Amplification of gene by PCR

By using an LA-PCR kit (manufactured by TaKaRa Brewery, Co.), PCR was performed under the following reaction conditions by using the genome DNA of B. subtilis strain BS as the template.

Reaction solution:

10 x buffer	5.0 microliter	
dNTP mix	8.0 microliter	
primer BS-1	2.5 microliter	(final concentration of 10 micro-M)
primer BS-2	2.5 microliter	(final concentration of 10 micro-M)
LA Taq polymerase	0.5 microliter	
genome DNA	1.5 microliter	(500 ng)
H_2O	30 microliter	
	50 microliter	

Amplification conditions:

a single cycle of 94 degrees for one minute;

30 cycles(, each cycle composed) of 94 degrees for one minute, 50 degrees for one minute and 72 degrees for two minutes.

After the termination of the reaction, the reaction solution was subject to agarose gel electrophoresis, and

the resulting amplified DNA fragment of about 1.3 kb was recovered by glass adsorption method. The fragment was ligated to a plasmid pT7Blue (manufactured by Novagen Co.) by using T4 DNA ligase according to a conventional method. By using then the ligation mixture, Escherichia coli (E. coli, JM109) was transformed. The resulting transformant was cultured in an LB culture medium. Subsequently, the bacteria were recovered, and from the recovered bacteria were extracted plasmids by using alkali/SDS method [Birnboim et al., Nucleic Acids Res., 7, 1513, 1979], followed by RNase A treatment, PEG (polyethylene glycol) precipitation, and deproteinization through phenol extraction and ethanol precipitation, to prepare plasmid DNA. The nucleotide sequence of the plasmid DNA thus prepared was determined by using A. L. F. DNA sequencer (manufactured by Pharmacia LKB Biosystems, Co.). The nucleotide sequence of the resulting DNA fragment is shown as SEQ ID NO.3 in the Sequence Listing. Because the amino acid sequences of the lysyl endopeptidase-digested fragments (No.2, 3, 4 and 5) (see Example 4) of the purified protein were found in the amino acid sequences anticipated from the DNA sequence, it was confirmed that the clone was a clone coding for a part of the objective arabinase.

(Example 6: Cloning of arabinase BS gene)

6.1 Southern blotting

250 ng of the genome DNA of B. subtilis strain BS was completely digested with restriction enzyme EcoRI, which was then subject to 1 % agarose gel electrophoresis. After electrophoresis, the gel was denatured (1.5 M NaCl/0.5 M NaOH for 45 minutes), followed by neutralization (1.5 M NaCl/1 M Tris-HCl (pH 8.0) for 30 minutes) and subsequent transfer of the separated DNA on a nylon film (Hybond N+, manufactured by Amersham, Co.), to immobilize the DNA under ultraviolet irradiation. The plasmid DNA containing the PCR fragments recovered in Example 5 was digested with restriction enzymes SpeI and AvaI, which were then subject to 1 % agarose gel electrophoresis, prior to recovery of a DNA fragment of about 1.3 kb by glass adsorption method, and the resulting fragment was used as a probe for Southern blotting. The probe was labeled with a fluorescent label by using a Fluorescein Gene Images labeling kit (manufactured by Amersham, Co.). Hybridization, rinsing and signal detection were conducted by using a Fluorescein Gene Images detection kit (manufactured by Amersham, Co.) according to the attached protocol. Consequently, two bands of about 0.5 kb and 1.5 kb were detected. Because it was believed that arabinase BS

gene was encoded in these bands, cloning was conducted thereafter.

6.2 Cloning of arabinase BS gene

The genome DNA of 10 microgram of B. subtilis strain BS was completely digested with a restriction enzyme EcoRI, which was subject to 1 % agarose gel electrophoresis, and then, the DNAs contained in fractions of 0.4 - 0.9 kb and 1- 2 kb were individually recovered by glass adsorption method. These DNA fragments were ligated to the EcoRI cleavage site of plasmid pBlueScript II SK+ (manufactured by Stratagene, Co.), and by using the resulting ligation mixture, E. coli JM109 was transformed. The DNA of the resulting transformant was immobilized on a nylon membrane, to screen positive clones by colony hybridization by using the PCR fragment of about 1.3 kb as recovered above in Example 5 as the probe. Consequently, clone A with an insertion fragment of about 0.55 kb was recovered from a library derived from the DNA eluted from the fraction of 0.4 - 0.9 kb, while clone B with an insertion fragment of about 1.5 kb was recovered from a library derived from the DNA eluted from the fraction of 1 - 2 kb.

5.3 Analysis of clones

The nucleotide sequences of the DNAs in the insertion fragments in the resulting two clones (clones A and B) were determined. On comparison with the sequence of the PCR fragment recovered in Example 5, it was indicated that clone A coded for the 5′ side while clone B coded for the 3′ side and that these clones were ligated together through the EcoRI cleavage site (SEQ ID NO.2, positions 280 - 285) on the genome DNA. A part of the nucleotide sequence is shown as SEQ ID NO.2. The DNA sequence had an open reading frame capable of coding for protein. Because the N-terminal sequence recovered by the amino acid sequence analysis of the purified protein and all the amino acid sequences of the lysyl endopeptidase-digested fragments (No.1 to No.6) were found in the amino acid sequence anticipated on the basis of the DNA sequence, it was confirmed that the DNAs contained in these clones coded for the objective arabinase. Herein, the N-terminus and the fragments of Nos.1 to 6 correspond to positions 1 to 20, positions 11 to 20, positions 155 to 181, positions 259 to 266, positions 416 to 427, positions 428 to 439 and positions 441 to 461, in this order, in the SEQ ID NO.1. By the screening of a protein with homology by using SWISS PROT data base, the protein exerted low homology (27 %) only with the sequence of the arabinofuranosidase of

Aspergillus niger (Flupphi, M. J. A. et al., Curr. Genet., 24, 525 - 532 (1993)), and therefore, it was confirmed on the basis of the amino acid sequence that the inventive arabinase (namely, arabinase BS) was novel.

6.4 Expression

Both two EcoRI fragments contained in the clones A and B were inserted in appropriate configurations into a multicopy vector pUS19 for E. coli, to construct plasmid pUC-BS, and by using the plasmid, E. coli strain DH5 alpha was transformed. As shown in Table 6 in Example 3, arabinase BS has alpha-L-arabinofuranosidase activity. Hence, the activity of arabinase BS in E. coli was confirmed by detecting alpha-L-arabinofuranosidase activity with a substrate p-nitrophenyl-alpha-L-arabinofuranoside. The strain was inoculated on an agar culture medium with the following composition for detecting arabinofuranosidase activity, for culturing at 37 degrees for 48 hours. For a colony of host E. coli introduced singly with pUS19, no color change occurred in the culture medium. For E. coli with pUC-BS introduced therein, alternatively, the periphery of the growing colonies turned yellow. Based on the outcome, it was confirmed that the cloned DNA was a

fragment coding for the objective enzyme with arabinofuranosidase activity.

Agar culture medium for detecting arabinofuranosidase activity

Bacto Tryptone	1 %
Bacto Yeast Extract	0.5 %
NaCl	0.5 %
p-Nitrophenyl alpha-L-arabinofuranoside (SIGMA Co.)	0.5 mM
Agar	1.5 %
Ampicillin	50 microgram/ml

(Example 7: Interaction between protopectinase and arabinase)

The interactive actions of the protopectinase and arabinase purified in Example 1 over the individual activities were examined.

Gradually increasing amounts of arabinase BS were added into protopectinase C at an amount corresponding to 0.4 unit of arabinase, to measure arabinase activity. The results are shown in Fig.1. Blank circle represents the total value of the arabinase activity of each of the two types of enzymes added; and solid circle represents the experimental value. As apparently shown in Fig.1,

the arabinase of protopectinase C together with the arabinase of arabinase BS exhibited a synergistic effect.

Then, gradually increasing amounts of arabinase BS were added into protopectinase C at an amount corresponding to 135 units of protopectinase, to measure protopectinase activity. The results are shown in Fig.2. Blank circle represents the total value of the protopectinase activity of each of the two types of enzymes added (arabinase BS does not exert protopectinase activity); and solid circle represents the experimental value. As apparently shown in Fig.2, the protopectinase activity of protopectinase C was enhanced by the addition of arabinase BS.

(Example 8: Effect of addition of soy bean powder extract solution)

An SP culture medium containing defatted soy bean powder (1.5 % soy bean powder (manufactured by Fuji Oil Industry)), 1.2 % KH_2PO_4, 2.8 % K_2HPO_4) was treated by using an autoclave at 121 degrees for 60 minutes, followed by extraction, and the resulting extract was used at the following procedures. Examination was made about the effect of the addition of the soy bean powder extract solution on arabinase BS, and the influence of

the concentration of the soy bean powder extract solution by means of rotary evaporator, boiling thereof for 10 minutes, and the dialysis thereof against distilled water on the aforementioned effect. The soy bean powder extract solution was added into a reaction solution to 0.1 % on a non-treated extract solution basis. As shown in Table 8, the soy bean powder extract solution enhanced the arabinase activity of arabinase BS. Additionally, the action was never influenced by the concentration by means of rotary evaporator or heating, but the action was eliminated due to the dialysis. In other words, it was indicated that a thermally stable low molecular substance in the soy bean powder extract solution had the arabinase activity enhancing action.

Table 8 Influence of a variety of treatments on arabinase activity enhancing action of soy bean powder extract solution

Additives (treatment method) (%)	Arabinase activity (units/ml)	Relative activity
Distilled water	370	100
Soy bean powder extract solution	610	165
Soy bean powder extract solution (evaporator)	650	175
Soy bean powder extract solution (evaporator/boiling)	730	195
Soy bean powder extract solution (evaporator/dialysis)	360	95

Then, gradually increasing amounts of the soy bean powder extract solution were added individually into protopectinase C and arabinase BS, to examine the effect thereof over the arabinase activities exerted by the individual enzymes. The results are shown in Fig.3. The soy bean powder extract solution enhanced the arabinase activity of any of the enzymes, and the effect

was strongly exerted over protopectinase C in particular.

The interactive actions of the three substances, namely protopectinase C, arabinase BS and soy bean powder extract solution, were examined. A variety of combinations of protopectinase C (corresponding to 0.3 unit of arabinase activity), arabinase BS (corresponding to 0.5 unit of arabinase activity) and soy bean powder extract solution (0.1 wt %) were added, to measure arabinase activity and protopectinase activity. As apparently shown in the results in Table 9, the three substances synergistically interacted with each other.

Table 9

Effect of soy bean powder extract solution over protopectinase activity and arabinase activity

Protopectinase (units/ml) Arabinase	Arabinase (units/ml) Protopectinase	Soybeen extract solution	Protopectinase (units/ml)	Arabinase (units/ml)
-	-	-	0	0
+	-	-	0	0.3
+	+	-	220	1.5
+	+	+	400	3.0
-	+	-	130	0.5
-	+	+	200	0.9

(Example 9: Scouring of cotton fiber)

In 5 ml of 0.1 % Warmin TE (manufactured by Tokai Chemical) in 20 mM phosphate buffer (pH 8.0) were added 100 mg of cotton yarn and a variety of combinations of protopectinase C (corresponding to 0.3 unit of arabinase activity), arabinase BS (0.5 unit) and soy bean powder extract solution (0.1 wt %), for treatment at 60 degrees for one hour. As a control, the cotton yarn was treated in 50 mM NaOH under the same conditions. The fibers after treatment were assessed of free pectin and tensile strength and at organoleptic tests. The tensile strength was measured by using Tensilon type tensile tester according to JIS-L-1095 (grasp interval of 20 cm and tensile velocity of 50 cm/min), and the mean values of ten measurements were calculated. Organoleptic tests were conducted over softness, gloss and touch at a 5-grade assessment mode by ten panelists, and the mean values were calculated. As shown in Table 10, excellent scouring was achieved by using the mixture of the aforementioned substances.

Table 10 Scouring of cotton fiber

Arabinase	Protopectinase	Soy been powder extract solution	Pectin (mg/g cotton fiber)	Strength (N)	Gloss	Softness	Touch
-	-	-	0.0	450	1.1	1.0	1.1
+	-	-	0.1	440	1.3	1.2	1.3
+	+	-	4.5	440	4.3	4.0	4.2
+	+	+	8.5	440	4.8	4.7	4.8
-	+	-	1.8	450	3.5	3.6	3.2
-	+	+	3.6	440	4.2	4.0	4.3
Control (NaOH)			4.4	300	4.1	3.3	3.2

(Example 10: Scouring of cotton cloth)

30 ml of Warmin TE was added into 30 liters of 20 mM phosphate buffer (pH 8.0), followed by thorough agitation and mixing and subsequent heating to 60 degrees. While keeping the temperature at 60 degrees, cotton cloth after preliminary desizing treatment was placed therein at a bath ratio of 30/1 (enzyme-buffer volume/cotton weight) and immersed therein for 15 minutes. Thereafter, 24 ml of protopectinase C (arabinase activity at 1000 units/ml), 12 ml of arabinase BS (arabinase activity at 1000 units/ml) and 30 ml of soy bean powder extract solution were added into the bath, for shaking and agitation at 60 degrees

for 15 minutes. After discharging the enzyme solution, the cotton cloth was rinsed five times in warm water at 60 degrees, and then, the cloth was dried.

Field-emission (FE) type scanning electron microscopic photographs of the cotton yarn drawn out from non-treated cotton cloth and the cotton yarn drawn out from the cotton cloth treated for pectin removal are shown in Figs.4 and 5. Primary wall was removed from the cotton yarn drawn out from the cotton cloth treated for pectin removal, where a secondary wall with micro-fibrils aligned and retained in a spiral shape developed, which indicates that the cotton cloth was excellent scoured.

Table 11 shows the pectin residue, wax residue and whitening degree of cotton cloths. Pectin residue in the treated cotton cloth was reduced to 1/2 fold the residue in the non-treated cotton cloth; as to wax residue, 80 % of the wax in the non-treated cotton cloth remained.

Herein, pectin was measured as follows. Cotton cloth after enzyme treatment was rinsed in water in warm water at 60 degrees until foam disappeared, and then, the cloth was dried until no weight reduction was observed at 60 degrees. From the dried cloth was cut out a test piece of a square shape of 5 cm x 5 cm, which

was then immersed in a preliminarily prepared Ruthenium Red staining solution (manufactured by Schimit, Gesershaft, Germany) at a concentration of 0.1 wt % and then left to stand at ambient temperature for 15 minutes. The staining solution should be prepared just prior to use. The stained test piece was rinsed in warm water at 60 degrees for 5 minutes, to remove excess staining solution. The test piece was dried at 60 degrees. The test piece was colored purple red. After pressing the test piece after drying at 60 degrees to flatten the remaining wrinkles, the test piece was irradiated with an integration sphere of a color analyzer (Type CA-3000, manufactured by Hitachi, Co.) under a light sourceD65 for spectrometry, to determine the reflectance R (D65). According to the formula by Kubelka-Munk (P. Kubelka, F. Munk, 1981), the absorption ÅEscattering coefficient K/S(lambda) was determined, and the value was defined as an optical concentration of wax deposited on fiber and cloth. The Kubelka-Munk formula is defined for specific wave length lambda as follows;

K/S(lambda) = (1-R(lambda)2) / 2R(lambda)

wherein K represents absorption coefficient and S represents scattering coefficient.

Wax residue was measured as follows. Cotton cloth after enzyme treatment was rinsed in water in warm water

at 60 degrees until foam disappeared, and was then dried until no weight reduction was observed. From the dried cloth was cut out a test piece of a square shape of 5 cm x 5 cm, which was then stained according to Waxoline Red Test Method (I. C. I., Method, Textile Laboratory Method, pp. 133 - pp. 134 (1975)). The test piece after staining was rinsed in cold water at 10 degrees, followed by dehydration, and subsequently, the cloth was retained at 110 degrees for 15 minutes, to develop color. The wax remaining on fiber and cloth was colored pink. The test piece was dried at 60 degrees. After pressing the wrinkles remaining on the test piece after drying to flatten the test piece, the test piece was irradiated with an integration sphere of a color analyzer (Type CA-3000, manufactured by Hitachi, Co.) under a light source D65 for spectrometry to determine the reflectance R (lambda). According to the formula by Kubelka-Munk (P. Kubelka, F. Munk, 1931), the absorption scattering coefficient K/S(lambda) was determined, and the value was defined as an optical concentration of wax deposited on fiber and cloth. The Kubelka-Munk formula is defined for specific wave length lambda as follows;

$$K/S(\lambda) = (1-R(\lambda)2) / 2R(\lambda)$$

wherein K represents absorption coefficient and S represents scattering coefficient.

Whitening degree was measured as follows.

Cotton cloth after enzyme treatment was rinsed in water in warm water at 60 degrees until foam disappeared, and was then dried until no weight reduction was observed at 60 degrees. From the dried cloth was cut out a test piece of a square shape of 5 cm x 5 cm, and after pressing the wrinkles remaining on the test piece after drying to flatten the test piece, the test piece was irradiated with an integration sphere of a color analyzer (Type CA-3000, manufactured by Hitachi, Co.) under a light sourceD65 for spectrometry, to determine color space parameters, Y, x, y, xa and ya. From these parameters are determined whitening degree W according to the following formula;

$$W = Y + 800\ (xa - x) + 1700\ (ya - y)$$

wherein

Y; Y value of three stimulation values of sample on XYZ color expression system

x, y; chromaticity coordinates on XYZ color expression system

xa, ya; chromaticity of cotton of complete dispersion and reflection type on XYZ expression system.

Table 11 Scouring of cotton cloth

Sample	Pectin residue (K/S)	Wax residue (X/S)	Whitening degree

Non-treated cotton cloth		
6.03	0.80	56.8
Scouring at 50 degrees		
2.98	0.68	57.5
Scouring at 60 to 95 degrees		
3.38	0.30	60.3

K/S (optical concentration); K represents photo-absorption and S represents photo-dispersion and reflection.
Whitening degree according to Kunter's formula; W = L - 3b.

(Example 11: Scouring of cotton cloth)

30 ml of Warmin TE was added into 30 liters of 20 mM phosphate buffer (pH 8.0), followed by thorough agitation and mixing and subsequent heating to 60 degrees. While keeping the temperature at 60 degrees, cotton cloth after preliminary desizing treatment was placed therein at a bath ratio of 30/1 (enzyme-buffer volume/cotton weight) and immersed therein for 15 minutes. Thereafter, 24 ml of protopectinase C (arabinase activity at 1000 units/ml), 12 ml of arabinase BS (arabinase activity at 1000 units/ml) and 30 ml of the soy bean powder extract solution were added

into the bath, for shaking and agitation at 60 degrees for 15 minutes. By further heating the enzyme-buffer solution up to 95 degrees, shaking and agitation were conducted for 15 minutes. After discharging the enzyme solution, the cotton cloth was rinsed five times in warm water, and then, the cloth was dried.

Field-emission (FE) type scanning electron microscopic photographs of cotton yarn drawn out from non-treated cotton cloth and the cotton yarn drawn out from the cotton cloth treated for pectin removal are shown in Figs.4 and 6. Primary wall was removed from the cotton yarn drawn out from the cotton cloth treated for pectin removal, where a secondary wall with micro-fibrils aligned and retained in a spiral shape developed, which indicates that the cotton cloth was excellent scoured.

Table 11 shows the pectin residue, wax residue and whitening degree of the cotton cloths. The pectin residue in the treated cotton cloth was reduced to 1/2 fold the residue in the non-treated cotton cloth; as to wax residue, 36 % of the wax in the non-treated cotton cloth remained. Although the reduction of wax was high, the whitening (bleaching) degree was improved.

(Example 12: scouring of linen fiber - ramie)

30 ml of Warmin TE was added into 30 liters of 20 mM phosphate buffer (pH 8.0), followed by gentle agitation and mixing so as to avoid foaming, and subsequently, the mixture was heated to 60 degrees. While keeping the temperature at 60 degrees, linen cloth after preliminary desizing treatment and the like was placed therein at a bath ratio of 30/1 (enzyme-buffer volume/cotton weight) and immersed therein for 30 minutes.

Thereafter, 24 ml of protopectinase C (arabinase activity at 3000 units/ml), 12 ml of arabinase BS (arabinase activity at 3000 units/ml) and 30 ml of the soy bean powder extract solution were added into the bath, for shaking and agitation at 60 degrees for 30 minutes. After discharging the enzyme solution, the linen cloth was rinsed five times in warm water, and then, the cloth was dried.

Scanning electron microscopic photographs of linen yarn drawn out from non-treated linen cloth and the linen yarn drawn out from the linen cloth treated for pectin removal are shown in Figs.7 and 8.

Primary wall was removed from the linen yarn drawn out from the linen cloth treated for pectin removal, where a secondary wall developed, which indicates that the linen cloth was excellent scoured.

(Example 13: scouring of linen fiber - linen)

30 ml of Warmin TE was added into 30 liters of 20 mM phosphate buffer (pH 8.0), followed by gentle agitation and mixing so as to avoid foaming, and subsequently, the mixture was heated to 60 degrees. While keeping the temperature at 60 degrees, linen cloth after preliminary desizing treatment and the like was placed therein at a bath ratio of 30/1 (enzyme-buffer volume/cotton weight) and immersed therein for 30 minutes.

Thereafter, 24 ml of protopectinase C (arabinase activity at 3000 units/ml), 12 ml of arabinase BS (arabinase activity at 3000 units/ml) and 30 ml of the soy bean powder extract solution were added into the bath, for shaking and agitation at 60 degrees for 30 minutes. After discharging the enzyme solution, the linen cloth was rinsed five times in warm water, and then, the cloth was dried.

Scanning electron microscopic photographs of linen yarn drawn out from non-treated linen cloth and the linen yarn drawn out from the linen cloth treated for pectin removal are shown in Figs.9 and 10.

Primary wall was removed from the linen yarn drawn out from the linen cloth treated for pectin removal,

where a secondary wall developed, which indicates that the linen cloth was excellent scoured.

Industrial Applicability

By using the composition composed of a combination of arabinase, protopectinase and soy bean powder extract solution in accordance with the present invention, the potency of these enzymes to treat plant tissues could be enhanced.

Since the composition of the present invention can reduce the cost for the treatment of plant tissues, including for example fiber scouring, pectin production and pulp production, the treatment can be practiced industrially.

SEQUENCE LISTING

SEQ ID NO:1:

LENGTH: 485

TYPE: amino acid

TOPOLOGY: linear

MOLECULE TYPE: protein

SEQUENCE

SEQ ID NO:2:

LENGTH: 1458

TYPE: nucleic acid

STRANDEDNESS: double

TOPOLOGY: linear

MOLECULE TYPE: genomic DNA

ORIGIN: name of biological organism; Bacillus subtilis
strain name; BS

SEQUENCE

SEQ ID NO:3:

LENGTH: 1316

TYPE: nucleic acid

STRANDEDNESS: double

TOPOLOGY: linear

MOLECULE TYPE: genomic DNA

ORIGIN: name of biological organism; Bacillus subtilis

strain name; BS

SEQUENCE

SEQ ID NO:4:

LENGTH: 20

TYPE: nucleic acid

STRANDEDNESS: single

TOPOLOGY: linear

MOLECULE TYPE: other nucleic acids, synthetic DNA

SEQUENCE

SEQ ID NO:5:

LENGTH: 20

TYPE: nucleic acid

STRANDEDNESS: single

TOPOLOGY: linear

MOLECULE TYPE: other nucleic acids, synthetic DNA

SEQUENCE

SEQ ID NO:6:

LENGTH: 30

TYPE: amino acid

TOPOLOGY: linear

MOLECULE TYPE: protein

SEQUENCE

Claims

1. An arabinase with the following properties;

(1) having an arabinan decomposition activity;

(2) having a molecular weight of about 65,000 on gels by SDS-polyacrylamide electrophoresis;

(3) having an optimum pH of 7.0;

(4) having an optimum temperature of 50 degrees; and

(5) being inhibited with Ag, Cd, Cu, Hg, Mn and Zn.

2. An arabinase according to claim 1, the arabinase being generated by Bacillus subtilis strain BS.

3. An arabinase according to claim 1, wherein the arabinase has an amino acid sequence of SEQ ID NO.1 or the arabinase has an amino acid sequence through deletion, substitution or addition of one or several amino acids from the amino acid sequence and has an activity at the same level as or a higher level than the level of the arabinase having the amino acid sequence of SEQ ID NO.1.

4. A composition containing an arabinase according to any of claims 1 to 3 and a protopectinase.

5. A composition according to claim 4, the composition

additionally containing a soy bean powder extract solution.

6. A composition according to claim 4, wherein the protopectinase has the following properties;

(1) having a protopectin decomposition activity;

(2) having a molecular weight of about 30,000 on gels by SDS-polyacrylamide electrophoresis;

(3) having an optimum pH of 6.0;

(4) having an optimum temperature of 60 degrees; and

(5) being inhibited with Hg and Mn.

7. A composition according to claim 4, wherein the protopectinase is generated by Bacillus subtilis strain BS.

8. A method for preparing a composition containing an arabinase and a protopectinase, comprising a process of culturing a microorganism of genus Bacillus, the microorganism generating the arabinase and the protopectinase, and a process of recovering a fraction containing the arabinase and protopectinase from the culture broth.

9. A method for scouring fibers, comprising a process

of reacting a composition according to any of claims 4 to 7 with fibers.

10. A method for producing pectin, comprising a process of reacting a composition according to any of claims 4 to 7 with fibers.

11. A method for producing pulp, comprising a process of reacting a composition according to any of claims 4 to 7 with fibers.

12. A DNA coding for an arabinase according to claim 3.

13. A DNA according to claim 12, wherein the DNA codes for an arabinase with the nucleotide sequence of SEQ ID NO.2 or with a nucleotide sequence generated through deletion, substitution or addition of one or several nucleotides from the nucleotide sequence and with an arabinase activity at the same level as or at a higher level than the level of the activity of the arabinase encoded by the nucleotide sequence of SEQ ID NO.2.

14. An expression vector carrying a DNA according to claim 12 or 13.

15. A host cell transformed with an expression vector according to claim 14.

16. A method for producing an arabinase, comprising a process of culturing a microorganism of genus Bacillus with the potency of generating the arabinase with the following properties;

(1) having an arabinan decomposition activity;

(2) having a molecular weight of about 65,000 on gels by SDS-polyacrylamide electrophoresis;

(3) having an optimum pH of 7.0;

(4) having an optimum temperature of 50 degrees; and

(5) being inhibited with Ag, Cd, Cu, Hg, Mn and Zn.

18. A production method according to claim 16, wherein the microorganism is Bacillus subtilis strain BS.

19. A method for producing an arabinase, comprising a process of culturing a host cell according to claim 15, wherein the arabinase has the following properties;

(1) having an arabinan decomposition activity;

(2) having a molecular weight of about 65,000 on gels by SDS-polyacrylamide electrophoresis;

(3) having an optimum pH of 7.0;

(4) having an optimum temperature of 50 degrees; and

(5) being inhibited with Ag, Cd, Cu, Hg, Mn and Zn.

19. A scouring composition containing arabinase, protopectinase and soy bean extract solution.

21. A method for scouring fibers, comprising a process of reacting an enzyme solution containing arabinase and protopectinase with fibers.

22. A method for scouring fibers according to claim 19, further comprising (the use of a) soy bean powder extract solution.

Fig.1

Arabinase activity (units)

Amount of arabinase BS added (units)

experimental values

calculated values

Fig.2

Protopectinase activity (units)

Amount of arabinase BS added (units)

experimental values

calculated values

Fig.3

Arabinase activity (units/ml)

Soy bean powder concentration (%)

Protopectinase C

Arabinase BS

Fig.7 Fig.9

non-treated

Fig.8 Fig.10

treated with enzymes